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# Southwestern corn borer damage and aflatoxin accumulation in conventional and transgenic corn hybrids

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## Abstract

Southwestern corn borer (*Diatraea grandiosella* Dyar) is a major pest of corn (*Zea mays* L.) in the southern United States. In addition to the direct yield losses caused by southwestern corn borer, larval feeding on developing ears provides a site for fungi to enter the ear. *Aspergillus flavus* Link: Fries infection and the subsequent accumulation of aflatoxin in corn grain are major limitations to profitable corn production in the southern United States. This investigation was conducted to determine the effectiveness of transgenic corn hybrids expressing the δ-endotoxin insecticidal (CryIAb) proteins isolated from *Bacillus thuringiensis* (*Bt*) in reducing southwestern corn borer damage and aflatoxin accumulation. Ear damage and aflatoxin accumulation were compared among 10 pairs of conventional non*Bt* and transgenic *Bt* corn hybrids following infestation with southwestern corn borer and inoculation with *A. flavus* using kernel-wounding and nonwounding techniques. Both non*Bt* and *Bt* hybrids exhibited high levels of aflatoxin accumulation when inoculated with a kernel-wounding technique. When inoculated with a non-wounding technique and infested with southwestern corn borer, aflatoxin accumulation was significantly higher in non*Bt* than *Bt* hybrids. Aflatoxin accumulation was also significantly higher for non*Bt* hybrids inoculated with *A. flav*us and infested with southwestern corn borer than for hybrids that were only inoculated with *A. flavus*. Southwestern corn borer larval establishment was significantly higher on non*Bt* hybrids than on *Bt* hybrids. Larval survival was extremely low on the *Bt* hybrids. The results of this investigation indicate that these *Bt* hybrids should be effective in reducing aflatoxin contamination in areas where high southwestern corn borer infestations occur. The reduced levels of aflatoxin accumulation associated with *Bt* hybrids are likely a consequence of reduced insect damage rather than resistance to *A. flavus* infection or aflatoxin accumulation per se.



*Keywords:* Aflatoxin; *Aspergillus flavus*; *Bacillus thuingiensis*; Maize; Southwestern corn borer

## 1. Introduction

Southwestern corn borer (*Diatraea grandiosella* Dyar) is a major insect pest of corn (*Zea mays* L.) in

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the southern United States and Mexico. Heavy infestations during the early vegetative stages of growth can reduce plant height and grain yield by more than half (Williams and Davis, 1984a, 1990). When larvae establish on plants after anthesis, they feed on leaf sheaths and tissues of developing ears before tunneling into the stalk (Davis et al., 1972). Although direct reductions in grain yield associated with southwestern corn borer infestations after anthesis are not as great as the reductions associated with infestations at earlier stages of plant growth, losses resulting from broken stalks and dropped ears can be substantial (Williams et al., 1983).

Larval feeding on developing ears also provides sites for fungi to enter, thus causing reduction in quality and value of grain produced (Davis and Williams, 1983). Increased *Aspergillus flavus* Link: Fries infection and the subsequent accumulation of aflatoxin are frequently associated with southwestern corn borer infestation of corn grown in the southern United States (Windham et al., 1999; Williams et al., 2002a,b). Aflatoxin is a naturally occurring toxin and a potent carcinogen (Castegnaro and McGregor, 1998). Aflatoxin is one of the major causes of hepatocellular carcinoma, the fifth most common cancer in the world (Wild and Hall, 2000). The presence of aflatoxin greatly reduces the value and marketability of corn. The U.S. Food and Drug Administration has set a tolerance of 20 ng $g^{-1}$ for aflatoxin $B_1$, the most common form of aflatoxin found in corn. Grain with higher levels of aflatoxin contamination is banned from interstate commerce (Gourma and Bullerman, 1995).

Host plant resistance is widely considered to be a desirable method of reducing losses from both southwestern corn borer and *A. flavus* as well as many other pests of corn. Considerable effort has been devoted to identifying and developing corn germplasm with resistance to damage by southwestern corn borer (Williams and Davis, 1989, 1997). Germplasm lines with moderate levels of resistance to leaf feeding by southwestern corn borer have been developed and released (Williams and Davis, 1982, 1984b, 2000). Some of the leaf feeding resistant lines also exhibit reduced ear damage from the pest. (Williams et al., 2002b).

Efforts to identify corn germplasm with resistance to infection by *A. flavus* and subsequent aflatoxin accumulation have been undertaken at several locations (Betran et al., 2002; Campbell and White, 1995; Scott and Zummo, 1988; Widstrom, 1996; Windham and Williams, 2002). Although germplasm with resistance to aflatoxin contamination has been identified, corn hybrids with high levels of resistance are not commercially available. Commercial corn hybrids do, however, differ in degree of susceptibility to aflatoxin contamination (Tubajeka et al., 2000; Windham and Williams, 1999).

Ear feeding insects significantly increase the incidence of molds and associated mycotoxins; however, the interactions are often not straightforward and involve many other environmental factors (Dowd, 1998). Although much effort has been devoted to identifying and developing corn germplasm with genetic resistance to insect damage in conventional breeding programs, technology for the transformation of corn provided new opportunities for enhancing resistance to insects. Tremendous effort has been expended on developing crop plants expressing genes that encode insecticidal proteins isolated from the bacterium *Bacillus thuringiensis* Berliner (Boulter, 1993). Transgenic corn plants expressing the δ-endotoxin insecticidal proteins proved to be highly effective against several species of Lepidoptera including southwestern corn borer (Armstrong et al., 1995; Williams et al., 1997, 1998, 1999). Use of *Bt* hybrids might be useful in reducing not only feeding of southwestern corn borer, but also associated losses from *A. flavus* infection and aflatoxin contamination. In an evaluation of five pairs of commercial *Bt*/non*Bt* corn hybrids, Williams et al. (2002c) reported that aflatoxin contamination was significantly lower in *Bt* hybrids than non*Bt* hybrids when infested with southwestern corn borer.

This investigation was undertaken to compare ear damage and aflatoxin accumulation among 10 pairs of conventional non*Bt* and transgenic *Bt* corn hybrids following infestation with southwestern corn borer and inoculation with *A. flavus*. Both kernel-wounding and non-kernel-wounding techniques were used to inoculate developing ears with *A. flavus.* A second objective of this investigation was to compare establishment and survival of southwestern corn borer larvae on *Bt* and non*Bt* hybrids.

## 2. Materials and methods

Conventional (non*Bt*) and transgenic (*Bt*) versions of 10 hybrid pairs with similar base genetics and adapted to the southern USA were provided by Monsanto Co., St. Louis, MO, in 2001 (Table 1) and 2002 (Table 2). Six pairs of hybrids were common to the two years. The hybrids were planted in a Leeper silty clay loam (fine, montmorillonitic, nonacid, thermic vertic Haplaquept) soil at Starkville, MS, on 20 April 2001 and 24 April 2002. The single-row plots were approximately 4 m long, spaced 1 m apart. Plots were overplanted and thinned to 20 plants. Standard corn production practices were followed. The hybrids were evaluated for aflatoxin contamination and ear damage caused by feeding of southwestern corn borer and other Lepidoptera in 2001 and 2002. The experimental design was a split-split-plot with five replications. Main plot treatments were four methods of *A. flavus* inoculation/southwestern corn borer infestation. Within main plots, hybrid base genetics was assigned to subplots and version (*Bt* or non*Bt*) to sub-subplots.

The following four methods of inoculation/infestation were used: (1) Approximately 14days after anthesis, a 3.4 ml suspension containing $3 \times 10^8$ *A. flavus* conidia in distilled water was injected underneath the husk into the side of the top ear using a tree marking gun (Zummo and Scott, 1989). Inoculum was prepared using *A. flavus* isolate NRRL3359 as described by Windham and Williams (1999). (2) Beginning when silks had emerged from 50% of the top ears of the earliest maturing hybrids, plants were inoculated weekly for five weeks with a suspension containing $9 \times 10^7$ conidia ml$^{-1}$ and a spreader sticker (Hi-Yield Chemical Co., Bonham, TX). The suspension was applied with a backpack sprayer (Solo, Newport News, VA) to the silks and husks of the top ear or ear shoot at the rate of 40 ml per plot. On the day following the second and third inoculations, each top ear was infested with 30 southwestern corn borer larvae by placing a mixture of larvae and corn cob grits in the axil of the top-ear leaf (Davis and Williams, 1997). Larvae were obtained from a laboratory colony maintained by the research unit. (3) Plants were inoculated weekly with an *A. flavus* conidial suspension as described above; however, ears were not

Table 1
Mean aflatoxin accumulation in 10 pairs of conventional (non*Bt*) and transgenic (*Bt*) corn hybrids following different methods of inoculation with *A. flavus* (AF) at Mississippi State in 2001

| Hybrid | | Aflatoxin | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Non*Bt* | *Bt* | Needle[a] | | AF spray + SWCB[bc] | | AF spray[b] | | Control[d] | |
| | | Non*Bt* (ng g$^{-1}$) | *Bt* (ng g$^{-1}$) | Non*Bt* (ng g$^{-1}$) | *Bt* (ng g$^{-1}$) | Non*Bt* (ng g$^{-1}$) | *Bt* (ng g$^{-1}$) | Non*Bt* (ng g$^{-1}$) | *Bt* (ng g$^{-1}$) |
| DK679 | DK679BTY | 1359 a | 893 a | 242 c | 199 abc | 608 a | 424 a | 131 a | 18 a |
| DK632 | DKC63-22 | 1344 ab | 852 a | 1116 ab | 124 abcd* | 261 ab | 179 abc | 72 a | 42 a |
| RX730 | RX730YG | 1238 abc | 1043 a | 1680 a | 264 ab* | 279 ab | 107 abc | 35 a | 89 a |
| DK647 | DK647BTY | 913 abcd | 885 a | 369 bc | 483 a | 474 a | 289 ab | 78 a | 17 a |
| DK626 | DK626BTY | 688 abcd | 556 ab | 36b c | 46 de* | 417 ab | 74 bcd | 8 a | 12 a |
| DK618 | DK618BTY | 627 abcd | 600 ab | 450 abc | 24 e* | 161 ab | 249 abc | 24 a | 13 a |
| DK697 | DKC69-70 | 589 bcd | 622 ab | 171 c | 334 ab | 156 ab | 186 abc | 14 a | 22 a |
| RX889 | RX889YG | 585 cd | 467 ab | 181 c | 63 cde | 155 ab | 246 abc | 15 a | 13 a |
| RX770 | RX770YG | 573 cd | 642 ab | 1140 ab | 38 de* | 116 b | 66 cd | 17 a | 4 a |
| DK687 | DKC68-70 | 499 d | 321 b | 309 bc | 92 bcde | 14 c | 21 d | 2 a | 3 a |

Means in a column followed by the same letter do not differ at $P = 0.05$. Tests of significance were performed on transformed [log($Y$ + 1)] means using Fisher's protected LSD before converting values back to the original scale.

[a] A spore suspension was injected into the side of the ear 7 days after silks had emerged from 50% of the plants in a plot.

[b] Beginning at 50% silk emergence, a spore suspension was applied to the husks and silks of the top ear weekly for 5 weeks using a backpack sprayer.

[c] At 7 and 14 days after silk emergence, 30 southwestern corn borer larvae were placed in the axil of the top ear leaf.

[d] Uninoculated, uninfested control.

* Significant difference between *Bt* and non*Bt* versions of the hybrid at $P = 0.05$.

Table 2
Mean aflatoxin accumulation in 10 pairs of conventional (non*Bt*) and transgenic (*Bt*) corn hybrids following different methods of inoculation with *A. flavus* (AF) at Mississippi State in 2002

| Hybrid | | Aflatoxin | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Non*Bt* | *Bt* | Needle[a] | | AF spray + SWCB[bc] | | AF spray[b] | | Control[d] | |
| | | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) |
| RX730 | RX730YG | 687 a | 246 bc* | 123a | 11 abc* | 29 a | 13 ab | 13 a | 2 a |
| DKC61-24 | DKC61-25 | 654 a | 639 a | 29 abc | 34 a | 8 a | 11 ab | 1 a | 2 a |
| DKC65-25 | DKC65-26 | 636 a | 371 abc | 9 bc | 2 bcd | 4 ab | 4 ab | 2 a | 7 a |
| DK647 | DK647BTY | 408 ab | 217 bc | 21 abc | 7 abcd | 13 a | 2 b | 3 a | 1 a |
| DK679 | DK679BTY | 390 ab | 400 abc | 27 abc | 1b cd* | 6 ab | 2 b | 8 a | 4 a |
| RX601 | RX601YG | 355 ab | 478 ab | 14 ab | 18 ab | 5 ab | 39 a | 9 a | 4 a |
| DK687 | DK68-70 | 346 ab | 197 bc | 3 c | 1 cd | 0 b | 4 ab | 2 a | 1 a |
| RX708 | RX708YG | 282 ab | 296 abc | 44 ab | 0 d* | 0 b | 2 b | 1 a | 1 a |
| RX889 | RX889YG | 275 ab | 181 c | 15 abc | 12 abc | 5 ab | 7 ab | 5 a | 1 a |
| DK697 | DK69-70 | 240 b | 61 d* | 40 ab | 1 cd* | 7 a | 1 b | 3 a | 4 a |

Means in a column followed by the same letter do not differ at $P = 0.05$. Tests of significance were performed on transformed [log($Y + 1$)] means using Fisher's protected LSD before converting values back to the original scale.

[a] A spore suspension was injected into the side of the ear 7 days after silks had emerged from 50% of the plants in a plot.

[b] Beginning at 50% silk emergence, a spore suspension was applied to the husks and silks of the top ear weekly for 5 weeks using a backpack sprayer.

[c] At 7 and 14 days after silk emergence, 30 southwestern corn borer larvae were placed in the axil of the top ear leaf.

[d] Uninoculated, uninfested control.

* Significant difference between *Bt* and non*Bt* versions of the hybrid at $P = 0.05$.

infested with southwestern corn borer larvae. (4) Plants were neither inoculated with *A. flavus* nor infested with southwestern corn borer larvae. However, neither fungicides nor insecticides were applied to prevent natural infection or infestation.

Mature ears were hand harvested approximately 60days after anthesis and dried for 7 days at 38 °C. Ear damage caused by feeding of southwestern corn borers or other Lepidoptera was visually scored on 10 ears from each plot on a scale of 1, no Lepidoptera damage, to 9, heavy damage (Williams et al., 2002a). Afterwards, ears from each plot were bulked and shelled. The grain was thoroughly mixed and ground using a Romer mill (Union, MO). Aflatoxin was determined in 50 g subsamples from each plot using the Vicam Aflatest (Watertown, MA).

An additional experiment was conducted in 2002 to provide information on establishment and survival of southwestern corn borer larvae on the *Bt* and non*Bt* hybrids. The hybrids were planted in a randomized complete block design with two replications in an area adjacent to the previously described experiment. Approximately 7 days after anthesis, 30 southwestern corn borer larvae mixed with corn cob grits were placed in the axil of the top-ear leaf using a mechanical larval dispenser (Davis and Williams, 1997). Fourteen days after infestation, the top ears were removed from 10 plants in each plot. Larvae were collected from each ear, identified by species, and counted.

Data for aflatoxin contamination were transformed by adding 1 and taking the logarithm of each number [log($Y + 1$)]. Plot means were calculated for each trait, and an analysis of variance was performed using PROC ANOVA (SAS Institute, 1987). Means were compared using Fisher's Protected LSD (Steel and Torrie, 1980).

## 3. Results and discussion

Overall aflatoxin levels were substantially higher in 2001 than in 2002. The overall mean for all hybrids and all treatments was 290 ng $g^{-1}$ in 2001 and 87 ng $g^{-1}$ in 2002. Aflatoxin accumulation was greatest when the hybrids were inoculated using the side needle technique (Zummo and Scott, 1989) in both 2001 and 2002 (Table 3). Among non*Bt* hybrids

Table 3
Mean aflatoxin accumulation in conventional (non*Bt*) and transgenic (*Bt*) hybrids following inoculation with *A. flavus* (AF).

| Inoculation method | Aflatoxin | | | |
|---|---|---|---|---|
| | 2001 | | 2002 | |
| | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) |
| Side needle[a] | 784 a | 637 a | 391 a | 261 a[**] |
| AB spray + SWCB[bc] | 417 b | 110 b[**] | 22 b | 5 b[**] |
| AF spray[b] | 192 c | 140 b | 5 c | 5 b |
| Control[d] | 24 d | 15 c | 4 c | 2 b |

Means in a column followed by the same letter do not differ at $P = 0.05$ (Fisher's protected LSD).
[a] A spore suspension was injected into the side of the ear 7 days after silks had emerged from 50% of the plants in a plot.
[b] Beginning at 50% silk emergence, a spore suspension was applied to the husks and silks of the top ear weekly for 5 weeks using a backpack sprayer.
[c] At 7 and 14 days after silk emergence, 30 southwestern corn borer larvae were placed in the axil of the top ear leaf.
[d] Uninoculated, uninfested control.
[**] Significant difference between *Bt* and non*Bt* hybrids at $P = 0.01$.

inoculated with this technique, aflatoxin levels ranged from 499 to 1359 ng $g^{-1}$ in 2001 (Table 1). Among the *Bt* hybrids, aflatoxin ranged from 321 to 1043 ng $g^{-1}$. Within their respective groupings, DK687 and its *Bt* version, DKC68-70, had the lowest levels of contamination. Earlier research indicated that DK687 is one of the less susceptible commercial hybrids currently available in the South (Windham and Williams, 1999). In 2002, aflatoxin levels ranged from 240 to 687 ng $g^{-1}$ among non*Bt* and 61 to 639 ng $g^{-1}$ among *Bt* hybrids (Table 2). Although differences between non*Bt* and *Bt* hybrids were not significant in 2001, differences between non*Bt* and *Bt* versions of two hybrids, RX730 and DK697, were significant in 2002 (Table 2). The non*Bt* versions of these two hybrids exhibited the highest and lowest levels of contamination, respectively.

Among the four treatments, the second highest levels of aflatoxin contamination within the non*Bt* hybrids resulted from the combination of *A. flavus* spray and southwestern corn borer infestation in both 2001 and 2002 (Table 3). The differences between non*Bt* and *Bt* hybrids for this treatment were highly significant both years. Each year the level of contamination was approximately 75% less for *Bt* hybrids than non*Bt* hybrids. This indicates a distinct advantage in planting *Bt* hybrids in areas where southwestern corn borer is a frequent and serious pest.

Further evidence of the role that southwestern corn borer can play in increasing aflatoxin contamination comes from a comparison of the effects obtained after the *A. flavus* spray inoculation alone and *A. flavus* spray in combination with southwestern corn borer infestation (Tables 1–3). Among the *Bt* hybrids, aflatoxin accumulation levels were not significantly increased by infesting plants with southwestern corn borer in either 2001 or 2002. With non*Bt* hybrids, however, aflatoxin contamination was significantly greater after southwestern corn borer infestation in combination alone. In 2002, only one *Bt* hybrid, DKC61-25, exceeded the 20 ng $g^{-1}$ limit imposed by FDA, but six of the non*Bt* hybrids exceeded that limit when infested with southwestern corn borer (Table 2).

Visual ratings of Lepidoptera damage to the ears after different inoculation/infestation treatments were made after mature ears were harvested, and it was not possible to distinguish damage caused by southwestern corn borer and other naturally occurring Lepidoptera. The side needle inoculation technique frequently results in injury to a few kernels of developing ears; this damage closely mimics damage from insect feeding. The visual ratings, therefore, represent damage caused by naturally occurring southwestern corn borer and other Lepidoptera and mechanical injury in addition to the damage caused by southwestern corn borer with which ears were artificially infested. Wounding, whether caused by insect feeding or the inoculation procedure, provides a potential site for *A. flavus* and other fungi to enter the ear.

In 2001, the mean ear damage rating for uninoculated/uninfested non*Bt* hybrids was significantly less than that of the non*Bt* hybrids after other treatments (Table 4). For the *Bt* hybrids, the damage rating was significantly higher for the side-needle-

Table 4
Mean insect damage ratings of ears harvested from 10 pairs of conventional (non*Bt*) and transgenic (*Bt*) corn hybrids following *A. flavus* (AF) inoculation and southwestern corn borer (SWCB) infestation in 2001 and 2002.

| Inoculation/infestation treatment | 2001 | | 2002 | |
|---|---|---|---|---|
| | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) |
| Side needle[a] | 4.0 ± 0.5 a | 3.8 ± 0.4 a | 4.5 ± 0.9 b | 3.4 ± 0.5 a** |
| AF spray + SWCB[bc] | 4.0 ± 0.6 a | 3.6 ± 0.6 b** | 4.9 ± 0.8 a | 2.9 ± 0.6 b** |
| AF spray[b] | 3.9 ± 0.5 a | 3.6 ± 0.5 b** | 3.8 ± 0.7 c | 2.9 ± 0.5 b** |
| Control[d] | 3.6 ± 0.5 b | 3.6 ± 0.5 b | 3.9 ± 0.7 c | 3.0 ± 0.6 b** |
| LSD (0.05) | 0.2 | 0.2 | 0.2 | 0.2 |

Ten ears from each plot were visually rated for damage caused by insect feeding on a scale of 0 (no damage) to 9 (heavy damage). Values represent means (±S.E.) of five replications of 10 hybrids. Means in a column followed by the same letter do not differ at $P = 0.05$.

[a] A spore suspension was injected into the side of the ear 7 days after silks had emerged from 50% of the plants in a plot.

[b] Beginning at 50% silk emergence, a spore suspension was applied to the husks and silks of the top ear weekly for 5 weeks using a backpack sprayer.

[c] At 7 and 14 days after silk emergence, 30 southwestern corn borer larvae were placed in the axil of the top ear leaf.

[d] Uninoculated, uninfested control.

** Significant differences between non*Bt* and *Bt* versions of the hybrids at $P = 0.01$.

inoculated hybrids than for the other treatments which did not differ. Differences between non*Bt* and *Bt* hybrids were highly significant for the *A. flavus* spray treatment and the *A. flavus* spray in combination with southwestern corn borer infestation. Differences between non*Bt* and *Bt* hybrids were not significant for the side needle inoculation or the uninoculated/uninfested control. This was probably because the side-needle inoculation of ears caused damage that was difficult to distinguish from insect feeding.

In 2002, the mean visual damage rating for non*Bt* hybrids was highest after the combined *A. flavus* spray inoculation and southwestern corn borer infestation. The side needle inoculation produced the second highest rating which was significantly greater than the *A. flavus* spray inoculation and the control. Among *Bt* hybrids, ear damage was significantly higher for the side needle than for all other treatments which did not differ. In 2002, when southwestern corn borer populations were high, visual ear ratings were

Table 5
Southwestern corn borer (SWCB) and corn earworm (CEW) larvae recovered from ears of conventional (non*Bt*) and transgenic (*Bt*) corn hybrids 14 days after infestation with 30 SWCB larvae per plant at Mississippi State in 2002

| Hybrid | | SWCB | | CEW | |
|---|---|---|---|---|---|
| Non*Bt* | *Bt* | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) | Non*Bt* (ng $g^{-1}$) | *Bt* (ng $g^{-1}$) |
| RX730 | RX730YG | 1.2 ± 0.4 bc | 0.1 ± 0.1 ab** | 0.4 ± 0.1 ab | 0.2 ± 0.0 bc |
| DKC61-24 | DKC61-25 | 0.3 ± 0.3 e | 0.0 ± 0.0 b** | 0.3 ± 0 ab | 0.3 ± 0.0 bc |
| DKC65-25 | DKC65-26 | 0.8 ± 0.1 d | 0.1 ± 0.1 ab** | 0.3 ± 0.4 ab | 0.5 ± 0.0 ab |
| DK647 | DK647BTY | 1.5 ± 0.6 a | 0.1 ± 0.0 ab** | 0.6 ± 0.2 a | 0.4 ± 0.1 bc |
| DK679 | DK679BTY | 0.7 ± 0.0 d | 0.2 ± 0.1 a** | 0.2 ± 0.2 b | 0.5 ± 0.1 ab* |
| RX601 | RX601YG | 1.3 ± 0.2 b | 0.1 ± 0.1 ab** | 0.1 ± 0.1 b | 0.1 ± 0.0 c |
| DK687 | DK68-70 | 0.7 ± 0.1 d | 0.0 ± 0.0 b** | 0.2 ± 0.1 b | 0.4 ± 0.1 bc |
| RX708 | RX708YG | 0.7 ± 0.1 d | 0.0 ± 0.0 b** | 0.3 ± 0.1 ab | 0.4 ± 0.1 bc |
| RX889 | RX889YG | 1.1 ± 0.1 c | 0.1 ± 0.0 ab** | 0.3 ± 0.3 ab | 0.5 ± 0.0 ab |
| DK697 | DK69-70 | 0.7 ± 0.1 d | 0.1 ± 0.1 ab** | 0.4 ± 0.1 ab | 0.8 ± 0.1 a* |
| LSD (0.05) | | 0.1 | 0.1 | 0.3 | 0.3 |
| Mean | | 0.9 | 0.1** | 0.3 | 0.4* |

Ears were removed from plants 14 days after infestation with 30 SWCB at 7 days after midsilk. Larvae were identified and counted. Means (±S.E.) were based on two replications of 10 plants.

* Significant differences between non*Bt* and *Bt* hybrids at $P = 0.05$.

** Significant differences between non*Bt* and *Bt* hybrids at $P = 0.01$.

significantly greater for non*Bt* than *Bt* hybrids for all treatments.

To aid in understanding the basis of the ear damage ratings, data on larval survival were collected from an additional experiment conducted in 2002. Fourteen days after plants were infested with 30 southwestern corn borer larvae, all Lepidoptera larvae were collected from the ears and husks of 10 plants from each plot. Larvae were not collected from other plant tissues. The mean number of southwestern corn borer larvae recovered from non*Bt* hybrids was 0.9, but only 0.1 from *Bt* hybrids. (Table 5). Differences in number of southwestern corn borer larvae recovered from *Bt* and non*Bt* versions were significant for all hybrid pairs.

Corn earworm [*Helicoverpa* zea (Boddie)] is another frequent pest of corn in the southern USA. Although plants were artificially infested with only southwestern corn borer, corn earworm larvae were present on the ears of both *Bt* and non*Bt* hybrids. The mean number of corn earworm larvae on ears of *Bt* hybrids (0.4) was significantly greater than on ears of non*Bt* hybrids (0.3). Although the *Bt* hybrids were highly effective against southwestern corn borer, they were not as effective against corn earworm. The slightly higher number of corn earworm larvae on *Bt* hybrids than non*Bt* hybrids might have resulted from the reduced competition with southwestern corn borer on *Bt* hybrids. It is also likely that visual ear damage ratings (Table 4) included significant amounts of damage caused by corn earworm. This would account for the significant ear damage sustained by all hybrids not infested with southwestern corn borer as well as the *Bt* hybrids infested with southwestern corn borer.

## 4. Conclusions

The results of this research indicate differences among hybrids in degree of susceptibility to aflatoxin contamination. The significantly higher levels of aflatoxin accumulation in non*Bt* hybrids than in *Bt* hybrids when plants were both inoculated with an *A. flavus* spray and infested with southwestern corn borer and an additional comparison with hybrids that received only the *A. flavus* spray inoculation indicate that southwestern corn borer can play a significant role in aflatoxin contamination of corn. Fortunately, these results also indicate *Bt* corn hybrids can be effectively used in reducing aflatoxin contamination. The high levels of aflatoxin accumulation in both *Bt* and non*Bt* hybrids after inoculation with the side needle technique indicate that the reduced levels of aflatoxin accumulation associated with *Bt* hybrids are likely a consequence of reduced insect damage rather than resistance to fungal infection or aflatoxin production per se. An inoculation technique such as the side needle, which wounds the ear, is not suitable for comparing aflatoxin contamination in *Bt* and non*Bt* hybrids. This technique masks the benefits of insect resistance. The *Bt* hybrids were highly effective against southwestern corn borer; however, both *Bt* and non*Bt* hybrids were naturally infested with corn earworm. Resistance to corn earworm would likely be an effective tool in reducing aflatoxin contamination in corn.

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